Minireview

The thermosome: archetype of group II chaperonins

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Abstract The thermosome, the chaperonin of the archaea, and its homologue from the cytosol of eukaryotes, known as TRiC or CCT, form a distinct subfamily of the chaperonins that does not depend on a co-chaperonin for protein folding activity. Recent structural data obtained by cryo- electron microscopy and X-ray crystallography provide the first insights into a novel mechanism remarkably different from that of the bacterial GroEL-GroES system.

Key words: Protein folding; Chaperone; TRiC/CCT; Archaeon

1. Introduction

Although Christian Anfinsen's dogma that the amino acid sequence of a polypeptide chain is sufficient to determine the three-dimensional fold of the mature protein still holds, it has become clear over the past decade that protein folding in vivo, i.e. in the crowded environment of the cell, is critically dependent on the assistance of chaperones, and in particular, of chaperonins (reviewed in [1]). Although their exact in vivo function is still under scrutiny [2,3], structural data and mutagenesis studies have dramatically advanced our understanding of the mechanistic aspects of these protein folding machines [4,5].

Until recently most structural studies on chaperonins have focused on a single member of this ubiquitous protein family, namely, GroEL of *Escherichia coli*. However, the results obtained with this particular system cannot necessarily be extrapolated to all chaperonins, especially not to those from archaea and the eukaryotic cytosol. These have been classified together as group II chaperonins in order to distinguish them from group I chaperonins, of which GroEL is the prototype. Group I chaperonins are found in eubacteria and their endosymbiotic descendants, mitochondria and chloroplasts [6]. An overview of the phylogeny of group II chaperonins is given in Fig. 1.

2. The thermosome – the chaperonin of the archaea

The first member of this second chaperonin family was discovered in the hyperthermophilic archaeon *Pyrodictium occultum* [7]. When *Pyrodictium* cells that had accidentally been exposed to a heat shock were examined by electron microscopy (EM), the lysed cells were observed to have released large amounts of a novel protein complex. At first glance the complex structurally resembled the well-known GroEL, and like GroEL it was heat shock-inducible and had ATPase activity. A more detailed analysis, however, revealed that the two rings each comprise eight subunits, and this suggested that this complex represents a novel type of chaperonin different from the sevenfold symmetric GroEL. Another peculiar feature of this novel chaperonin is its heterooligomeric nature; two equally abundant subunits, α and β, were found in the complex and were proposed to alternate in position within the two rings. The heat shock induction and the extreme temperature profile of this ATPase led to the name thermosome [8].

3. TRiC/CCT – the chaperonin of the eukaryotic cytosol

Shortly thereafter, a similar chaperonin-like ATPase called TF55 (thermophilic factor of 55 kDa) was found in the archaeon *Sulfolobus shibatae* and shown to be related to the eukaryotic t-complex polypeptide [9], which had been implicated in microtubule function [10]. TCP-1 is now known to be one of the eight different but related subunits of the eukaryotic cytosolic chaperonin [11] called TRiC (TCP-1 ring complex [12]) or CCT (chaperonin containing TCP-1 [13]). TRiC/CCT is essential for the folding of several proteins, most prominently the cytoskeletal proteins tubulin [14] and actin [15]. It is likely that the divergence of TRiC/CCT subunits occurred very early in the evolution of eukaryotes, presumably in parallel with the evolution of the eukaryotic cytoskeleton. The evolution of exactly eight gene families [16] seems to have been determined by the fact that eight subunits can be accommodated in each of the two rings and that each type of subunit occupies a distinct position [17]. Nevertheless, multicellular eukaryotes may possess additional, facultatively expressed subunits that in certain tissues replace the constitutive subunits [13,18].

4. Thermosome composition and subunit arrangement

The heterooligomeric nature of TRiC/CCT is prefigured by the two subunits that make up thermosomes in many archaea, for example in *Pyrodictium occultum*, *Thermoplasma acidophilum* or *Sulfolobus acidocaldarius* [7] as well as in *Sulfolobus solfataricus* [19], *Thermococcus* [20] and *Haloferax volcanii* [21]. Also for TF55, originally considered to be a homooligomer [9], a second subunit called TF56 has now been identified [22]. In contrast, only one thermosome gene is present in the genome of *Methanococcus jannaschii* [23]; and also the thermosomes from *Methanopyrus kandleri* [24], *Pyrococcus* sp. [25], *Desulfurococcus* sp. [26] and *Archaeoglobus fulgidus* [7] appear to be homooligomers.

Regardless of whether they are built from one, two or eight different subunit types, group II chaperonins are mostly hexa-

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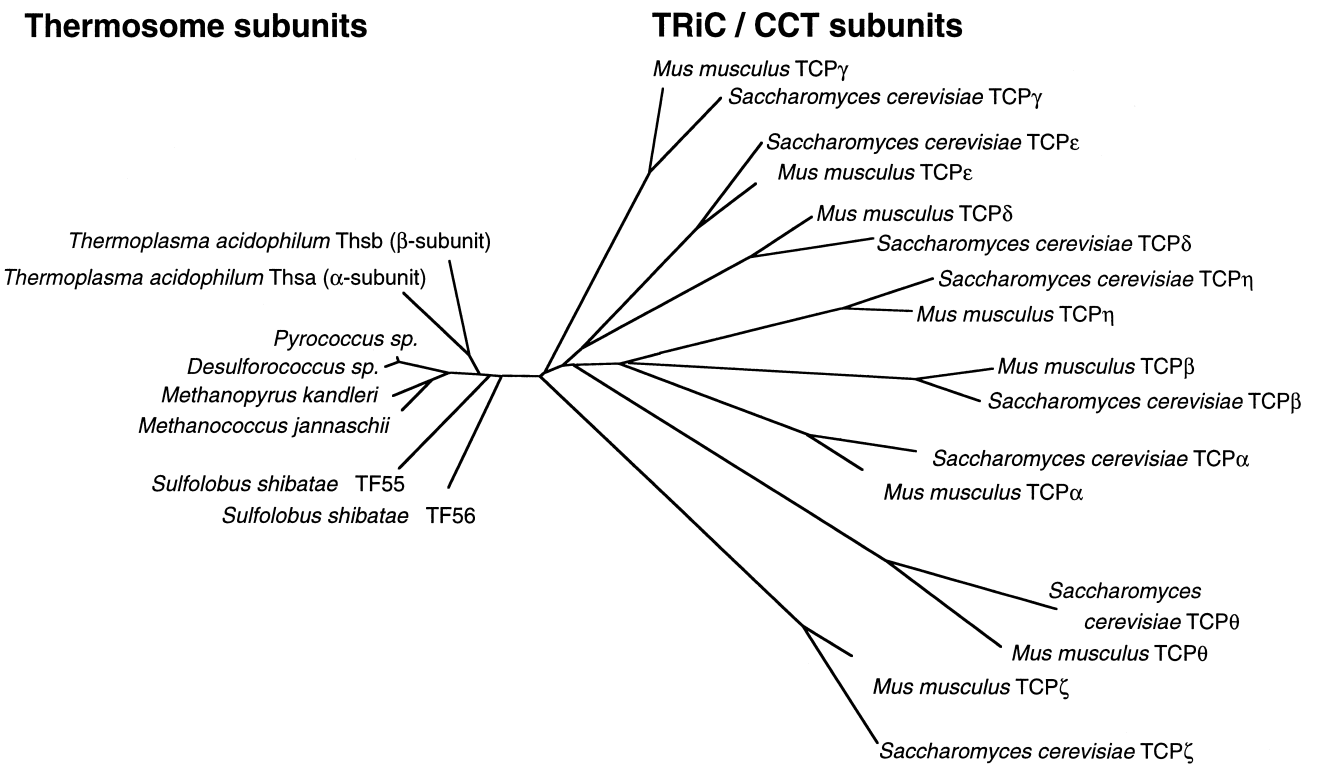

Fig. 1. Dendrogram of the group II chaperonins (for details see Nitsch et al. [28]).

decamers. The only known exceptions occur in *Sulfolobus* in which octadecameric chaperonins seem to be the rule. Three independent methods of image analysis have shown that chaperonins purified from *Sulfolobus solfataricus* are exclusively composed of nine-membered rings [27]. Heterologous expression of the α subunit of the *Thermoplasma acidophilum* thermosome alone yields a subset of particles with nine-fold symmetry (5%) in addition to a majority of particles with eight-fold symmetry [28]. This demonstrates that no major structural changes are required for a transition from eight- to nine-membered rings.

The arrangement of the two subunits in heterooligomeric thermosomes was determined by cryo-EM [28], which revealed a four-fold symmetry in heterooligomeric thermosomes from *Thermoplasma acidophilum*, but eight-fold symmetry in recombinant, α-only thermosomes. This not only proved that the original proposal of alternating subunits in two identical rings [7] was correct but also corresponds to the subunit arrangement found in the eukaryotic cytosolic chaperonin TRiC. In the latter, both rings contain all eight subunits, which occupy distinct positions [17]. The alternative model, which postulates that heterooligomeric group II chaperonins generally are composed of different homooligomeric rings [22], can explain neither the four-fold symmetry in the *Thermoplasma* thermosome nor the co-precipitation of all TRiC/CCT subunits. The subunit arrangement of the nine-membered rings of octadecameric *Sulfolobus* chaperonins remains enigmatic, the more so since there are conflicting reports of equimolar [22] or 2:1 stoichiometry [19] of the two subunits.

5. Thermosome function

Upon examination by EM, thermosomes are sometimes observed to form fibers or rafts of fibers (Pfeifer and Baumeister, unpublished), as do a variety of other proteins such as RNA polymerase, glutamine synthetase and the chaperonin GroEL from *E. coli* [29]. Similar observations led Trent et al. [30] to suggest that thermosomes serve as building blocks of an archaeal cytoskeleton. However, in the absence of in vivo evidence other than the abnormal cytoskeleton of mutants in TCP-1, that can be fully accounted for by the dependence of both tubulin and actin folding on TRiC/CCT, this remains entirely speculative. The only solidly established functions are the binding [9,31] and the folding [20,32,33] of denatured polypeptides, both of which have been demonstrated for the thermosome. For TRiC/CCT, even more extensive proof for a function in protein folding has accumulated (reviewed in [34,35]).

Until recently, very limited structural information on group II chaperonins was available, and therefore their mechanism remained poorly understood. Most puzzling was the absence in both the archaea [23] and the eukaryotic cytosol [36] of a homologue for GroES, the essential co-chaperonin of GroEL. GroES can bind to the same residues in the apical domain of GroEL that are responsible for binding substrate protein [37,38]. Under the influence of ATP, GroEL undergoes a conformational change which results in and is reinforced by the binding of GroES [39]. The latter displaces substrate from the binding sites into the central cavity and simultaneously serves as a lid to create a closed folding compartment with a hydrophilic inner surface. Thus, substrate can fold without interference by other proteins in what is called the Anfinsen cage [40].

The lack of a co-chaperonin is reflected in the primary sequences of group II chaperonins, which are similar to those of group I chaperonins only in their N- and C-terminal parts

[6,41]. In the crystal structure of GroEL [42], these form the equatorial domain that contains the ATP binding site and mediates inter-subunit contacts. In contrast, the middle region of the sequences that forms the apical domain responsible for substrate (and GroES) binding, shows no significant similarity between the two groups. Therefore it remained unclear whether the apical domain of group II chaperonins had a fold similar to that of GroEL or whether its structure differed in order to allow a co-chaperonin-independent mechanism. This unsettled issue prompted us to determine the structure of the substrate binding domain of the thermosome α subunit by X-ray crystallography [43].

6. Thermosome apical domain

The core of the thermosome apical domain resembles the apical domain of GroEL, but lacks the hydrophobic residues implicated in binding substrates to group I chaperonins. Rather, a large hydrophobic surface patch is found in a novel helix-turn-helix motif that is characteristic of all group II chaperonins and most likely contains the substrate binding site. This protrusion is clearly set apart from the main body of the apical domain and is not found in GroEL or other group I chaperonins. Comparison with the GroEL apical domain indicates that this novel feature is incompatible with binding of a GroES-like cofactor. Upon analysis of two different crystal forms of the thermosome apical domain, we found that the helical protrusion is highly flexible and can be tilted at least 20° relative to the globular core of the apical domain. Therefore, group II chaperonins seem to contain a third hinge region in addition to the two identified in group I chaperonins [39].

7. Thermosome conformations

By combination of X-ray and cryo-EM data, we have been able to put forward an initial model for the orientation of the apical domain in the ground state of the holochaperonin [43]. Similar to the ring formed by the substrate binding sites of GroEL [42,44], the hydrophobic protrusions are located at the inner rim of the central cavity, where bound substrates have been mapped on the level of the apical domains [28]. The iris-like arrangement of the protrusions suggested that a closing movement of the complex would bring them into a position where they occlude the opening of the central cavity [43] (Fig. 2). This explained why a GroES-like co-chaperonin is not needed to seal off the folding compartment of group II chaperonins. After complex closure, the hydrophobic surfaces of the protrusions would be blocked by preferential interaction with each other [43], thus mimicking the binding of GroES to the substrate binding site of GroEL [38]. In group II chaperonins, therefore, a single polypeptide chain provides both GroEL and GroES functions as has been suggested previously [8].

More recently, a crystal structure of the complete thermosome [45] has confirmed both the alternating arrangement of α and β subunits [28] and our proposal that the substrate binding protrusion also serves as a functional equivalent of GroES [43]. In contrast to the open, substrate-accessible conformation of the chaperonins seen on cryo-EM, the crystal structure of the thermosome shows a closed conformation, in which the novel protrusions (also called lid domains [45]) indeed occlude the central cavity of the chaperonin (Fig. 3). Furthermore, the central cavity is mostly hydrophilic, consistent with the notion that this occluded compartment is the Anfinsen cage where protein folding takes place. Because ATP can be observed in the structure when soaked into the crystals, crystallization conditions appear to have forced the chaperonin into a conformation resembling the ATP-liganded state. Unexpectedly, in the closed conformation one of the two helices in the substrate binding protrusion switches to a β-strand conformation and forms a β-barrel with its equivalents on other subunits in the ring. The unusual capability of this polypeptide segment to adopt both helical and sheet conformations and the flexibility of its attachment to the remainder of the apical domain (see above) may have evolved in order to broaden the range of substrates accommodated by the thermosome.

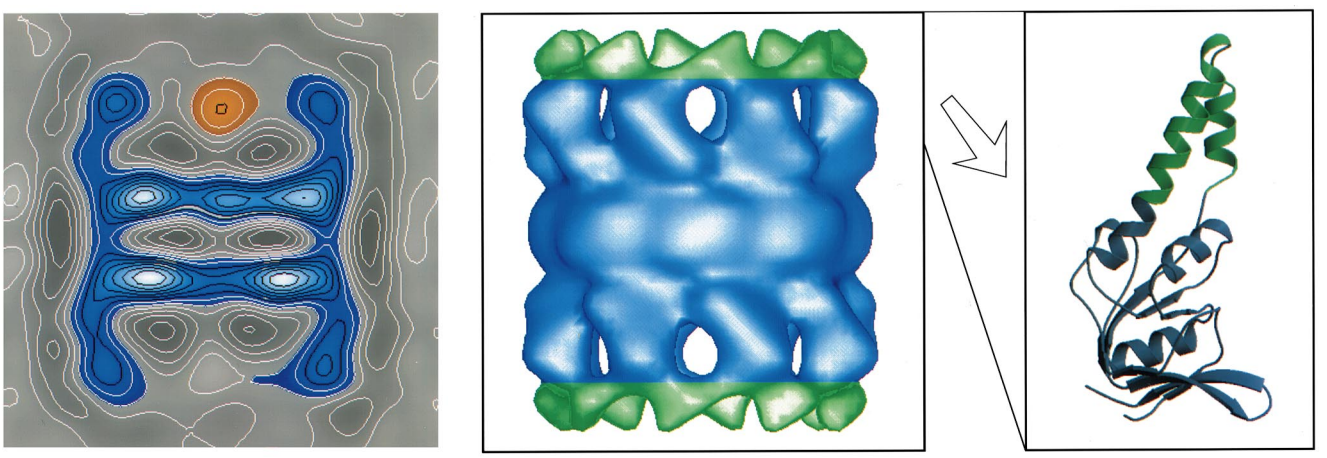

Fig. 2. Structure of the thermosome from *Thermoplasma acidophilum*. Left: EM-based projection map (side view) of the ice-embedded complex. Density from the thermosome is represented in blue; density assigned to substrate in gold (for experimental details see Nitsch et al. [28]). Middle: Three-dimensional reconstruction of the *Thermoplasma* thermosome obtained by cryo-electron tomography (for experimental details see Walz et al. [53]). The complex is in an open, substrate binding conformation. The protrusion which acts as an iris-type lid allowing closure of the central cavity is color-coded in green. Right: Ribbon diagram of the apical substrate binding domain determined by X-ray crystallography. The helix-turn-helix protrusion is shown in green (for experimental details see Klumpp et al. [43]).

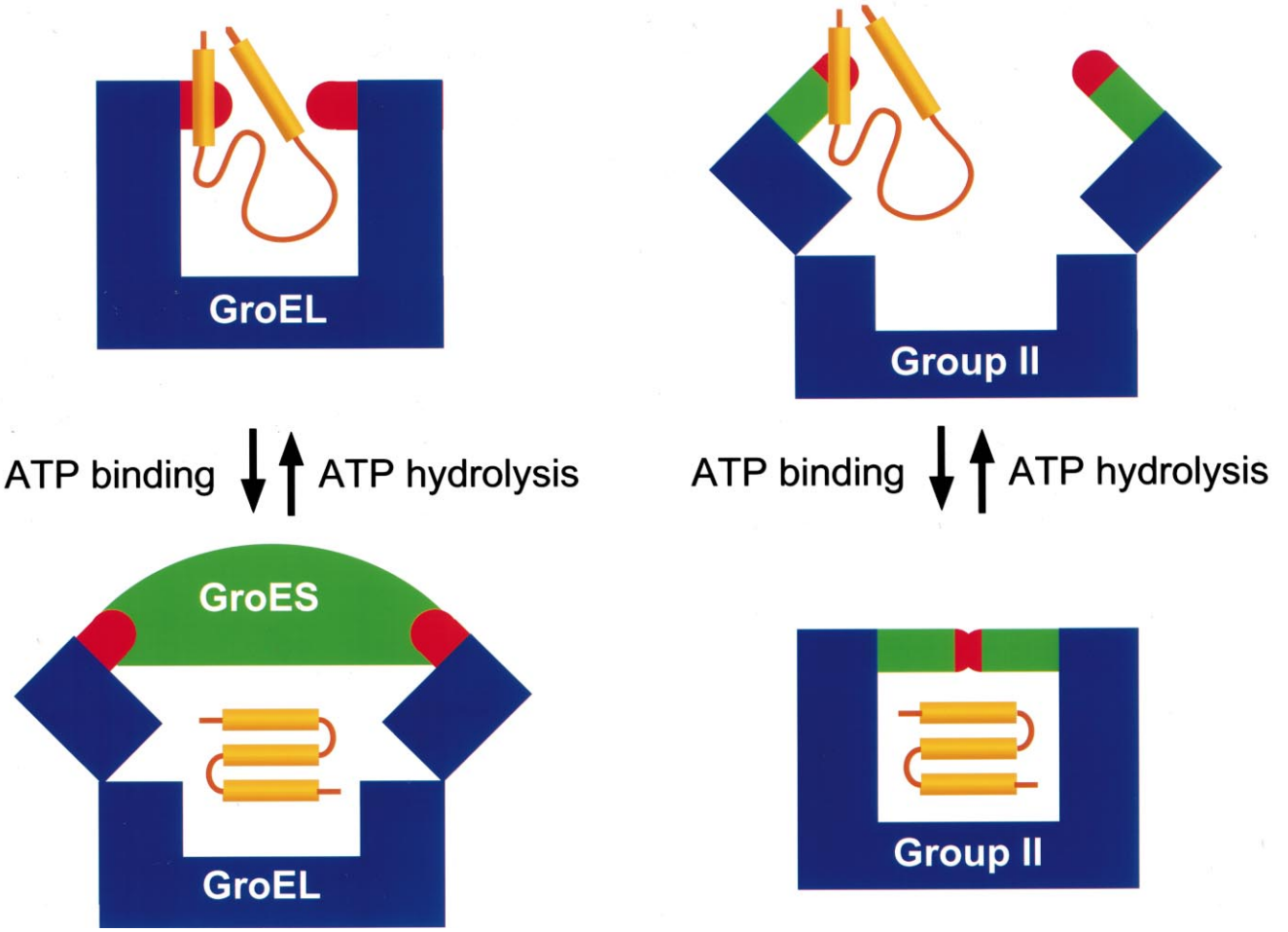

Fig. 3. Comparison of the basic conformations of group I (GroEL-GroES) and group II chaperonins within the functional cycle. For the sake of simplicity the schematic drawings of the chaperonin-substrate complexes show only single rings. The color coding corresponds to that in Fig. 2. For details see text.

8. Functional cycle of group II chaperonins

The two thermosome conformations observed by cryo-EM [28] and X-ray crystallography [45] most likely represent the two basic states that form the functional cycle of group II chaperonins. The conformational changes observed by tryptophan fluorescence upon ATP binding to the thermosome [32] probably correspond to the transition between these two states. In the absence of K^+, which is required for hydrolysis, the ATP-liganded thermosome cannot bind new substrate [32]. Similar effects are observed for TRiC/CCT after incubation with ADP and BeF, which jointly mimic the transition state of ATP hydrolysis [46]. On the other hand, upon incubation with the non-hydrolyzable ATP analog AMPPNP, α-tubulin previously bound to TriC/CCT undergoes at least partial folding without discharge from the chaperonin [47]. Presumably, ATP binding drives group II chaperonins from the open, substrate binding conformation into the closed conformation where substrate folds within the central cavity. Hydrolysis of ATP would then allow the chaperonin to return to the open conformation with subsequent release of folded substrate. Alternatively, Joachimiak and coworkers [48] have proposed transient dissociation of the complex in order to release bound substrates. However, in their model, substrate folds in association with an 'open' conformation, whereas the functional cycle proposed here assumes folding to occur in a closed, cage-like conformation equivalent to the GroEL-ES complex.

The model we propose here is consistent with sedimentation velocity measurements which show an expansion of TRiC/CCT upon tubulin binding but contraction of the chaperonin in the presence of ATP [49]. It is also consistent with EM images of negatively stained TRiC/CCT particles with and without addition of ATP [50], although the full extent of the ATP-induced conformational changes will only be revealed by cryo-EM. Furthermore, the proposed transition from an open conformation with exposed hydrophobic protrusions to the closed conformation with occluded substrate binding sites can easily explain the finding that ATP prevents proteolytic cleavage within the protrusions of TRiC [51].

Although the functional consequences of ATP binding and hydrolysis for folding of polypeptide substrate seem to have been conserved between the catalytic cycles of group I and group II chaperonins, the effects of nucleotides on the overall structure of the two chaperonin groups apparently differ. The substrate binding ground state of the thermosome observed by cryo-EM is open [28] and contracts upon ATP binding to yield the closed conformation seen in the crystal structure [45]. In contrast, GroEL binds its substrates in a compact conformation, and ATP causes its apical domains to move outward

to allow binding of GroES and thus closure of the cavity. At present, the reason for this difference is unclear. Possibly, the absence of an equivalent to helices H11 and H12 of GroEL reorients the apical domains of the group II chaperonins such that they respond differently to the forces generated by the switch between ADP and ATP states of the equatorial domains. In order to further analyze the ATP-induced domain movements, a detailed structural description of the open conformation is required. As extensive crystallization trials both in the presence and in the absence of nucleotides have only yielded crystals of the closed thermosome conformation [45], the most promising method to derive the exact domain orientations in the ground state is to fit the atomic structures of the individual domains to density maps obtained by cryo-EM [52]. Three-dimensional reconstructions performed by tomography in sensu strictu [53] have the advantage that three-dimensional density maps of individual particles can be subjected to image classification. Thus, one can distinguish different conformations that may co-exist in a population of chaperonin molecules.

Although the elucidation of these and other structural details will certainly lead to further refinement of the model presented here, a general outline of the group II catalytic cycle has now been established. This provides new opportunities for functional studies, particularly by site-directed mutagenesis. Therefore, the thermosome and TRiC/CCT may soon cease to be second-class chaperonins.

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